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# Proliferative dependent regulation of the glyceraldehyde-3-phosphate dehydrogenase/uracil DNA glycosylase gene in human cells

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**The relationship between the proliferative dependent expression of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH)/uracil DNA glycosylase (UDG) gene and the induction of uracil DNA glycosylase activity was examined in human cells. Three different cell types were studied to determine whether the growth-dependent regulation of this multifunctional gene was a common characteristic of human cells. These included WI-38 normal embryonic lung fibroblasts, a Japanese Bloom's syndrome non-transformed skin fibroblast cell strain (GM-05289) and a lymphoblastoid cell line transformed by the Epstein–Barr virus. The Japanese Bloom's syndrome cells displayed the altered immunoreactivity with marker monoclonal antibody 40.10.09 which characterizes cells from this human genetic disorder. In non-cycling human cells Northern blot analysis using a plasmid (pChug 20.1) which contained the human GAPDH/UDG cDNA revealed a single 1.6 kb transcript. In each case, the expression of this gene was increased during cell proliferation. This increase in GAPDH/UDG gene expression was identical to that observed for UDG enzyme activity. Further, using anti-human UDG monoclonal antibodies, there was a growth-dependent rise in immunoreactivity suggesting an increase in the level of antigenic protein. These results demonstrate that: (i) the expression of the GAPDH/UDG gene was dependent on the proliferative state of the cell; and (ii) a correlation existed between the transcription of this gene and the level of uracil DNA glycosylase enzyme activity.**

## Introduction

Human cells contain two major DNA excision repair pathways, nucleotide excision and base excision, which recognize and remove lesions perturbing DNA structure and which miscode during DNA replication (1). Each step in the pathway is catalyzed by a specific protein which is transcriptionally regulated. Recent evidence demonstrated that this regulation of human DNA repair pathways was dependent on the proliferative state of the cell (1). This has been defined by growth-related increases in enzyme activity (2–9), DNA repair synthesis (10–12) and by an increase in the excision rates of DNA adducts (13–16). The increase in DNA repair during the cell cycle may function to prepare DNA for replication by removing DNA damage resulting from spontaneous DNA degradation or from exposure to physical and chemical agents.

The base excision repair enzyme uracil DNA glycosylase (UDG*) catalyzes the removal of uracil from DNA by the hydrolysis of the N-glycosylic bond between the base and the deoxyribose (1). Uracil can be found in DNA either through the direct incorporation of dUTP instead of dTTP during DNA synthesis (17) or by the mutagenic deamination of cytosine residues (18). The enzyme has been purified from a number of procaryotic and eucaryotic organisms. The mammalian nuclear glycosylase is a monomer of Mr=33–37 kDa (19–21). UDG activity was increased during mammalian cell proliferation in a defined temporal sequence with respect to DNA replication (3,4). A series of anti-human placental UDG monoclonal antibodies were prepared (22) and shown to specifically recognize the 37 kDa protein (21,23). A 1.3 kb UDG cDNA was isolated by using these monoclonal antibodies to immunoscreen a human placental cDNA library in lambda gt11 (24). It encoded a 1.6 kb mRNA which directed the synthesis of the 37 kDa protein. Recently, sequence analysis demonstrated a complete homology of this cDNA with that reported for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (25). Further analysis demonstrated that the 37 kDa monomer of this protein exhibited UDG activity (25).

The current study was initiated to consider the relationship between the transcription of this gene encoding a multifunctional protein in relation to the proliferative dependent regulation of a very specific and seemingly unique DNA repair enzyme. Further, GAPDH has been used as a marker in studies examining eucaryotic gene regulation based on the presumption that its expression was constitutive. For these reasons, the regulation of the human GAPDH/UDG gene was examined in diverse human cell types. We now report that the transcriptional expression of the human GAPDH/UDG gene was proportional to the proliferative state of the cell and that there was a correlation between GAPDH/UDG gene activity and the level of UDG enzyme activity. These results define the growth regulation of this gene as a common characteristic in human cells and demonstrate a direct relationship between that expression and the levels of UDG activity.

## Materials and methods

### *Cell culture*

Normal human fibroblast cell strains WI-38 and CRL 1222 (ATCC, Rockville, MD) and Bloom's syndrome fibroblast cell strain GM-05289 (NIGMS Human Cell Repository, Camden, NJ) were maintained in 150 $cm^2$ tissue culture flasks. The cells were fed twice a week with low glucose DMEM supplemented with 10% fetal calf serum, 100 U penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine. Cells were incubated at 37°C in a 5% $CO_2$ atmosphere. Cells were split 1:2 at confluence. Molt cells were a generous gift of Dr Earl Henderson. They were cultured in 150 $cm^2$ flasks in RPMI-1640 (Gibco) supplemented with 1% pen-strep, 2% glutamine and 10% fetal calf serum. Cells were fed 3 days/week by removing 10 ml of medium and the addition of 10 ml of fresh medium. Molt cells were split by removing 10 ml of medium and dividing the remaining 5 ml equally into two flasks. Fresh media (12.5 ml each flask) was added and cells were cultured as described.

To initiate proliferation in non-transformed cells, confluent cells were harvested with trypsin/EDTA and plated at a concentration of $1 \times 10^5$ cells per 100 mm dish. In Molt cells, cells in spent media were resuspended at an initial concentration of $2 \times 10^6$ cells/$T_{150}$ flask and allowed to proliferate. In each cell type, cells

*Abbreviations: UDG, uracil DNA glycosylase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

were collected at 24 h intervals. The rate of DNA synthesis was measured by a 30 min [$^3$H]thymidine pulse (30 μCi/10 ml medium, 2 Ci/mmol). Cells were collected by centrifugation; the pellets were resuspended in 0.5 ml of buffer I (20 mM Tris–HCl, pH 8.0, 1 mM dithiothreitol, 20% glycerol) and then sonicated for 20 s at 60 watts on ice. Cell debris was removed by centrifugation at 2300 *g* for 10 min at 4°C. TCA insoluble material was collected on glass fiber filters and the radioactivity determined in a liquid scintillation counter. Cell number was determined by averaging cell counts in three 100 mm dishes per time point.

*Determination of UDG activity*

The specific activity of the uracil DNA glycosylase was determined by *in vitro* biochemical assay. Crude cell extracts were prepared from human cells as described (3,4). Protein was determined by the Bradford method (26). UDG activity (c.p.m./μg of total protein) was quantitated by the release of [$^3$H]uracil from a poly(dA)-poly($^3$HdU) substrate in a 100 μl reaction consisting of: 100 mM Tris–HCl (pH 8.0), 10 mM $K_2$EDTA, 5 mM dithiothreitol, 100 μg BSA, 1 μg of the polynucleotide substrate (1925 c.p.m./pmol) and 1–20 ng of total cell protein. The mixture was incubated for 30′ at 37°C; terminated by the addition of 300 μl ethanol (−20°C), 100 μl of 1 mg/ml heat denatured calf thymus DNA, and 60 μl of 2 M NaCl; then stored overnight at −20°C. The precipitate was collected by centrifugation at 2300 *g* for 10 min at 4°C; radioactivity (200 μl aliquot) in the ethanol soluble supernatant was determined by liquid scintillation spectroscopy.

*ELISA analysis*

Glycosylase immunoreactivity was determined by ELISA as previously described (27,28). Briefly, ELISA was performed in quadruplicate with each anti-human placental uracil DNA glycosylase monoclonal antibody over the indicated range of protein concentrations. Each sample was bound for 2 h at 37°C followed by a 48 h incubation at 4°C. Alkaline phosphate-conjugated F(ab′) fragment of sheep antiserum to mouse IgG was used as the second antibody. A 1:250 dilution was bound to the plate for 2 h at 37°C. For detection of alkaline phosphatase activity, p-nitrophenyl phosphate (1:20 dilution, 50 μl total volume) was added and the reaction allowed to proceed overnight at room temperature in the dark. Colorimetric analysis was performed using a Bio-rad model 2550 EIA reader.

*RNA isolation*

Total RNA was isolated from confluent non-transformed fibroblast monolayers grown in 150 cm$^2$ tissue culture flasks by the method of Chirgwin *et al.* (29). Cells were lysed in the flasks by the direct addition of 1 ml of guanidinium isothiocyanate extraction buffer (4 M guanidinium isothiocyanate containing 0.1 M Tris–HCl (pH 7.6) and 1% 2-mercaptoethanol). Nuclear DNA in the lysate was sheared by repeated passage through a 25 gauge needle. Cesium chloride was added to the lysate (1 g/2.5 ml lysate) and the lysate was layered onto a cushion of 5.7 M CsCl in 4 mM $Na_2$EDTA (pH 7.5). Total RNA was pelleted by centrifugation at 42 000 r.p.m. for 16 h at 20°C. RNA pellets were dissolved in sterile deionized-distilled water and precipitated overnight by the addition of 0.1 vol of 3 M sodium acetate (pH 5.2) and 2.5 vol of cold ethanol. The RNA precipitate was collected by centrifugation at 10 000 *g* for 30 min. The pellet was dissolved in sterile water and the RNA stored as a precipitate. For Northern blot analysis 15 μg of total RNA was denatured and electrophoresed on a 1.5% agarose gel containing 6.6% formaldehyde using MOPS electrophoresis buffer. The gel was prepared for transfer to nylon by soaking for two 20 min periods in 10 × SSC. The RNA was crosslinked to the membrane by exposure to short wave UV (254 nm). The membrane was prehybridized in a sealed bag for 2 h with 50% deionized formamide, 0.5 × Denhardt's solution, 5 × SSPE (0.9 M NaCl, 0.05 M $NaPO_4$ pH 7.7, 5 mM $Na_2$EDTA), 0.1% SDS, 0.2 mg/ml denatured salmon sperm DNA and 10% dextran sulfate at 40°C. Heat denatured $^{32}$P-labelled pChug 20.1 (5 × 10$^5$ c.p.m. per lane) was added and the membrane was hybridized for 12–24 h. Following hybridization the membrane was washed under moderate stringency: two 20 min washes in 1 × SSC, 0.1% SDS at room temperature, followed by two 30 min washes in 0.1 × SSC, 0.1% SDS at 55°C.

## Results

Two criteria were used initially to define the uracil DNA glycosylase in the Japanese Bloom's syndrome cell strain. First, the reactivity of the native, non-denatured enzyme was determined by ELISA using three different anti-human placental UDG monoclonal antibodies. Recent evidence demonstrated that each antibody recognized a determinant on the multifunctional 37 kDa subunit of human GAPDH (Seal *et al.*, submitted for publication). The Bloom's syndrome enzyme was immunoreactive in a concentration dependent manner with two antibodies, 37.04.12 and 42.08.07 (Figure 1A). However, this protein was not recognized by the marker monoclonal antibody, 40.10.09. Using

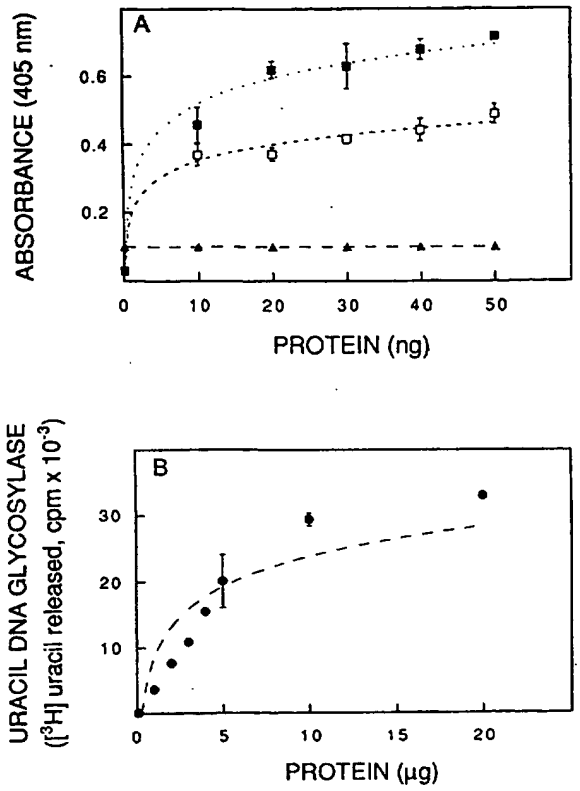


**Fig. 1.** Characterization of the uracil DNA glycosylase in confluent GM-05289 Bloom's syndrome cells. Crude cell extracts were prepared as described in Materials and methods. Immunoreactivity and catalytic activity were determined by ELISA and *in vitro* biochemical assay, respectively. ELISA was performed with each anti-human placental uracil DNA glycosylase monoclonal antibody over the indicated range of crude protein concentrations. UDG activity was quantitated using 1–20 ng of total cell protein. Error bars denote experimental variability. Symbols without error bars describe experiments where statistical error is the size of the symbol. (**A**) ELISA reactivity of Bloom's syndrome GM-05289 uracil DNA glycosylase. (-□-) monoclonal antibody 42.08.07, (-■-) monoclonal antibody 37.04.12, (-▲-) Bloom's syndrome marker monoclonal antibody 40.10.09. (**B**) Activity of the Japanese Bloom's syndrome uracil DNA glycosylase.

that antibody, no ELISA reactivity was observed at any of the protein concentrations which were examined.

To consider whether the altered immunoreactivity of the Bloom's syndrome enzyme reflected a reduction of the level of glycosylase activity, the specific activity of the uracil DNA glycosylase in confluent GM-05289 cells was then examined. Enzyme activity was quantitated using the identical cell free sonicates utilized in the ELISA. The specific activity of the glycosylase, 2.07 pmol/mg total protein (Figure 1B), was similar to that found in normal cell strains and in other Bloom's syndrome cells (30). These results demonstrate that although the specific activity of the enzyme was equivalent to that observed in normal human cells, a Japanese patient with Bloom's syndrome was also characterized by the immunological alteration detected by marker monoclonal antibody 40.10.09.

The expression of the GAPDH/UDG gene in normal human and Bloom's syndrome cells was then examined. Immunoblot analysis demonstrated that the 40.10.09 antibody detected a single 37 kDa protein (Figure 2A). Accordingly, the altered immunoreactivity of the Bloom's syndrome glycosylase was not due to a loss of the primary amino acid sequence which comprises the 40.10.09 antigenic determinant. Similarly, Northern blot analysis

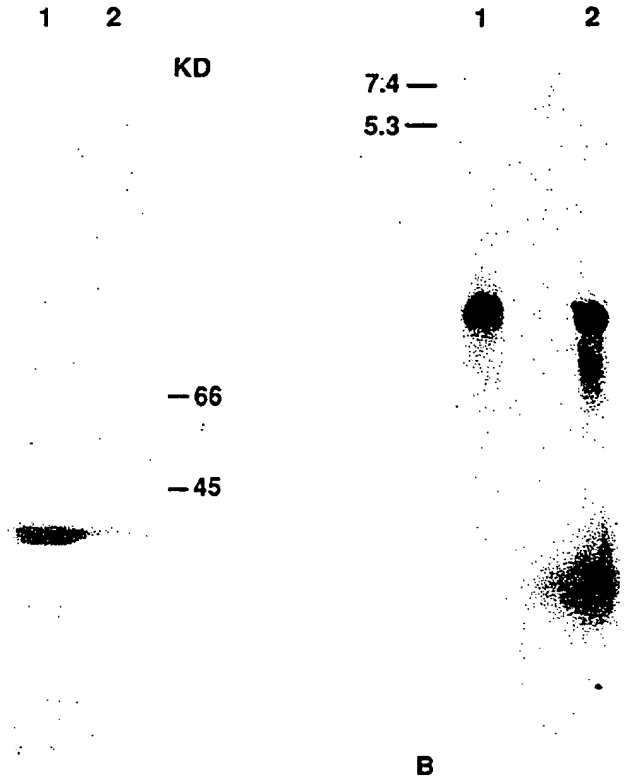


Fig. 2. Expression of the GAPDH/UDG protein and gene. (A) For Western blot analysis, 50 μg of purified human placental uracil DNA glycosylase and crude cell-free sonicate from Bloom's syndrome cell strain GM-05289 were electrophoresed in a 10% polyacrylamide−SDS gel (37) then electroblotted to nitrocellulose (38). Reactive proteins were detected using monoclonal antibody 40.10.09 (27,28). The migration of the molecular weight markers is indicated on the left. Lane 1, human placental uracil DNA glycosylase; lane 2, crude cell sonicate from confluent GM-05289 fibroblast cultures. (B) For Northern blot analysis total RNA (15 μg) were electrophoresed in a formaldehyde denaturing gel (6.6% formaldehyde, 1.5% agarose) and transferred to nylon by capillary action with 10 × SSC. The blot was hybridized with $^{32}$P-labeled pChug 20.1 probe (1 × $10^6$ c.p.m., 4 × $10^8$ c.p.m./μg plasmid DNA). Lane 1, Human placental RNA; lane 2, GM-05289 RNA. The migration of standard DNA (lambda *Hin*dIII digest) is shown on the left.

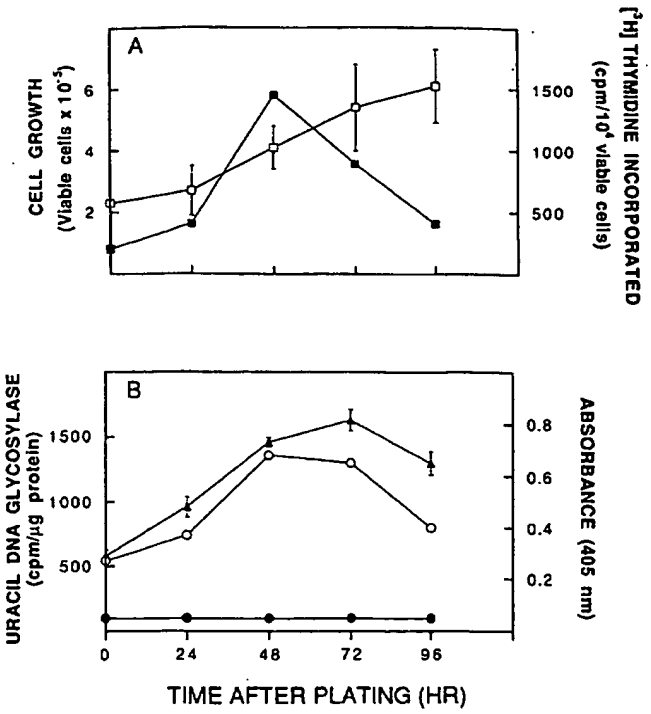


Fig. 3. Regulation of the GAPDH/UDG protein in Bloom's syndrome cells. Immunoreactive GAPDH/UDG protein was determined using 50 ng of total protein from each time point for ELISA with monoclonal anti-uracil DNA glycosylase antibodies 42.08.07 and 40.10.09. Cell growth was determined by calculating the mean number of viable cells from three cultures. Viable cells (-□-) were quantitated by trypan blue dye exclusion. DNA synthesis (-■-) was measured by [$^3$H]thymidine incorporation. Uracil DNA glycosylase activity/μg protein (-▲-) was quantitated as described in Materials and methods. ELISA with monoclonal antibody 42.08.07, -○-, and marker monoclonal antibody 40.10.09, -●-. Error bars denote statistical variability as defined in the legend to Figure 1.

using the pChug 20.1 plasmid containing the 1.3 kb GAPDH/UDG cDNA demonstrated that this probe hybridized to a single RNA size class of approximately 1.6 kb (Figure 2B, lane 1). A similar RNA hybridizing band was observed using total RNA from normal human fibroblasts (Figure 2B, lane 2). As the level of intensity appeared to be comparable in both human cell strains this would suggest an equivalent level of uracil DNA glycosylase gene expression in non-cycling normal human and Bloom's syndrome cells.

The expression of the Bloom's syndrome GAPDH/UDG protein was then examined during cell proliferation. Confluent cells were replated in fresh medium at a lower density to initiate cell proliferation. Cell number started to increase at 48 h and plateaued at the 72−96 h interval (Figure 3A). At that period of the growth curve, a 2.5-fold increase in the number of cells was observed. As measured by [$^3$H]thymidine incorporation, the rate of DNA replication started to increase at 24 h, reached its maximum at 48 h and declined at the later intervals. An 8-fold increase in the rate of DNA synthesis was observed at the 48 h interval.

The regulation of the uracil DNA glycosylase was then examined in parallel cultures. As measured by *in vitro* biochemical assay, the increase in cell number was accompanied by an increase in UDG enzyme activity (Figure 3B). Glycosylase activity started to increase at 24 h, was maximally induced 2.6-fold at 48−72 h, and then decreased at the 96 h interval. Using the identical cell samples, the increase in the level of immunoreactive GAPDH/UDG protein was examined by ELISA. As shown in Figure 3B, there was an increase in immunoreactivity when the glycosylase monoclonal antibody 42.08.07 was used in the ELISA. The 2.9-fold increase in immunoreactivity observed at 72 h was comparable to the 2.6-fold increase in biochemical activity. However, a different result was observed when the 40.10.09 monoclonal antibody was used. In this instance no immunoreactivity was observed in any of the cell samples over the entire growth curve. This result demonstrates an increased level of antigenic GAPDH/UDG protein which increased in extent and temporal sequence to that observed for the increase in UDG enzyme activity. Further, they reveal that this growth-related protein contained the identical immunological defect observed in the protein present in non-cycling cells.

The proliferative dependent expression of the normal human and the Bloom's syndrome GAPDH/UDG gene was then examined in relation to the increase in UDG enzyme activity (Figure 4). The level of GAPDH/UDG mRNA at each time point was quantitated as the amount of hybridized radioactive pChug 20.1/mg of RNA. In normal human cells, GAPDH/UDG transcription increased during cell proliferation (Figure 4A). At 48 h there was a 5.8-fold increase in GAPDH/UDG gene expression. As cell growth diminished at 96 h, GAPDH/UDG transcription was diminished. This pattern of gene regulation paralleled the sequence of UDG enzyme activity which was

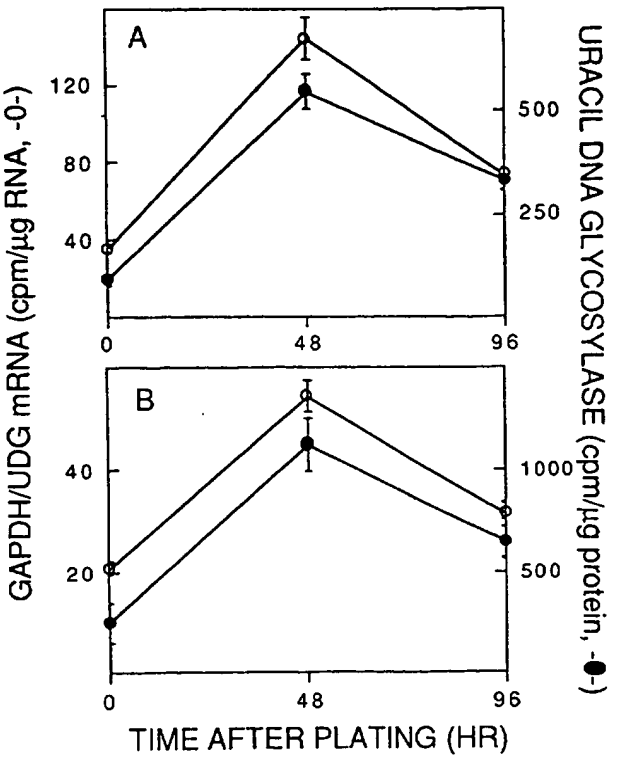


**Fig. 4.** Transcriptional expression of the GAPDH/UDG gene during cell proliferation. RNA was prepared at the indicated intervals as described in Materials and methods. Each point represents the mean of triplicate hybridizations. UDG activity was quantitated as described in Materials and methods. Experimental variation was determined as described in the legend to Figure 1. (**A**) Normal human CRL-1222 cells, (**B**) Japanese Bloom's syndrome GM-5829 cells.

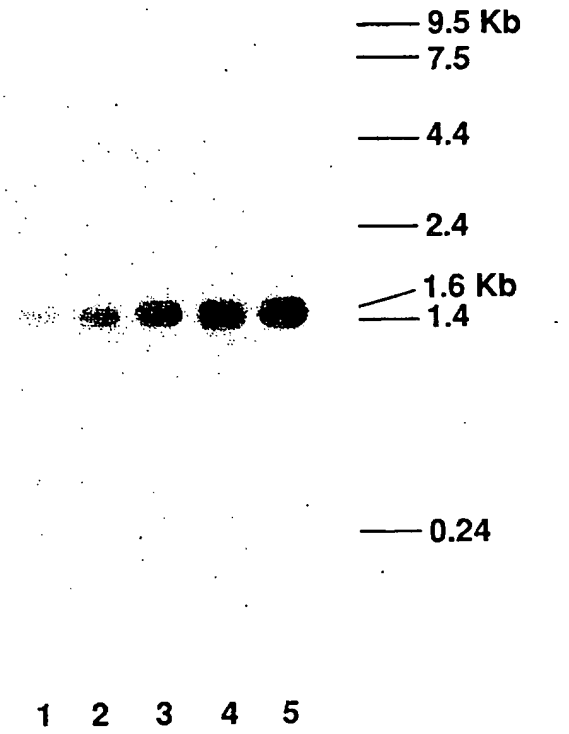


**Fig. 5.** Transcriptional expression of the GAPDH/UDG gene in human lymphoblastoid cells. Molt cells were cultured and harvested as described in Materials and methods. Total RNA was isolated according to the method of Chomczyski and Sacchi (39). Molt cells ($1 \times 10^6$ cells) were treated with $RNA_{zol}$ (200 μl), extracted with chloroform (0.1 vol), and precipitated with isopropanol (1 vol). RNA was washed twice with 75% EtOH and dissolved in 1 mM $Na_2EDTA$ (pH 7.0). Northern blot analysis was performed as described in Materials and methods using 10 μg of RNA. Lanes 1–5 correspond to RNA from days 0–4 after replating.

increased 4-fold at 48 h and was then diminished at 96 h. A similar pattern was observed in the Bloom's syndrome cells (Figure 4B). At 48 h, GAPDH/UDG gene expression was increased 3.8-fold with transcriptional activity reduced at 96 h. This was comparable to the 2.6-fold rise in UDG enzyme activity observed in these cells. These results demonstrate a defined relationship between the level of transcriptional expression of the GAPDH/UDG gene and the activity of the UDG in asynchronously proliferating normal human and Bloom's syndrome cells.

As a number of studies indicated that viral transformation can alter the expression of DNA repair genes (31–34), the proliferative-dependent regulation of the GAPDH/UDG gene was examined in an Epstein–Barr virus transformed human lymphoblastoid cell line. Northern blot analysis demonstrated that the pChug 20.1 plasmid containing the 1.3 kb human GAPDH/UDG cDNA hybridized to a single 1.6 kb RNA species (Figure 5, lane 1). As cell growth was initiated, there appeared to be a proliferative-dependent increase in the extent of hybridized radiolabeled plasmid to the 1.6 kb RNA species (Figure 5, lanes 1–5, normalized to RNA concentration). The relative rate of GAPDH/UDG gene transcription in the Molt cell line was examined by densitometry. Maximal levels were observed at the 96 h interval after cell growth was initiated (Figure 6, lane 5). Furthermore, this analysis also demonstrated a consistent increase in the level of GAPDH/UDG mRNA during cell growth.

The induction of cell growth and enzyme activity were monitored in parallel cultures to consider their relationship to GAPDH/UDG gene expression. Cell number increased steadily (Figure 7A) while the rate of DNA synthesis was maximal at 48 h and then declined somewhat at later intervals. Quantitation of the UDG demonstrated a continual increase in enzyme activity (Figure 7B) which was directly parallel to increased mRNA levels observed for the GAPDH/UDG gene (Figure 7B).

## Discussion

In this report, we provide evidence that the regulation of the multifunctional GAPDH/UDG gene was dependent on the proliferative state of the cell. Three criteria were used to define the dependence of GAPDH/UDG expression on cell growth. First, Northern blot analysis demonstrated the selective increase in the level of GAPDH/UDG mRNA, identifying a specific increase in gene transcription. Second, ELISA defined an increase in the amount of antigenic protein during cell proliferation, suggesting that the biosynthesis of the GAPDH/UDG protein paralleled gene expression. Third, the induction of UDG enzyme activity correlated with the increase in transcriptional regulation and the synthesis of antigenic protein. As increased GAPDH/UDG gene expression was observed in three disparate human cell types, it seems likely that such regulation during cell growth is a common characteristic of human cells. This is in accord with a number of findings demonstrating growth related increases in a number of DNA metabolic enzymes and genes (1). Furthermore, this finding is especially noteworthy as it has been suggested that the GAPDH gene may be used as a marker gene on the presumption that its expression is constitutive. Accordingly, the GAPDH/UDG

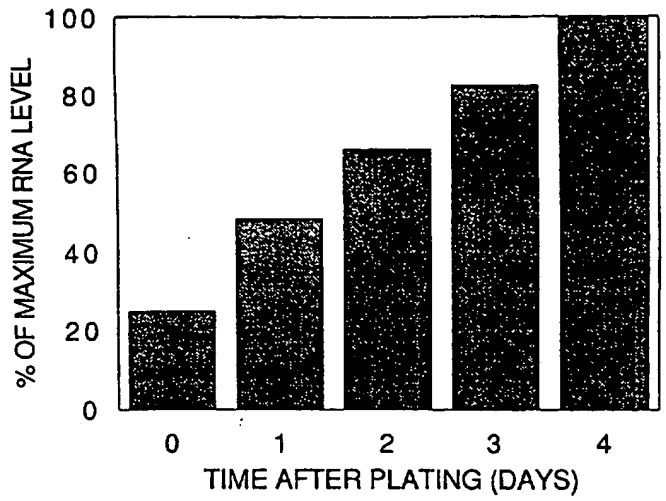


**Fig. 6.** Densitometric analysis of GAPDH/UDG RNA levels in proliferating Molt cells. The intensity of each band in Figure 5 was determined with a Biorad Model 1650 scanning densitometer. RNA levels at each time point are given as percentage values at the maximally enhanced levels which were observed on day 4.

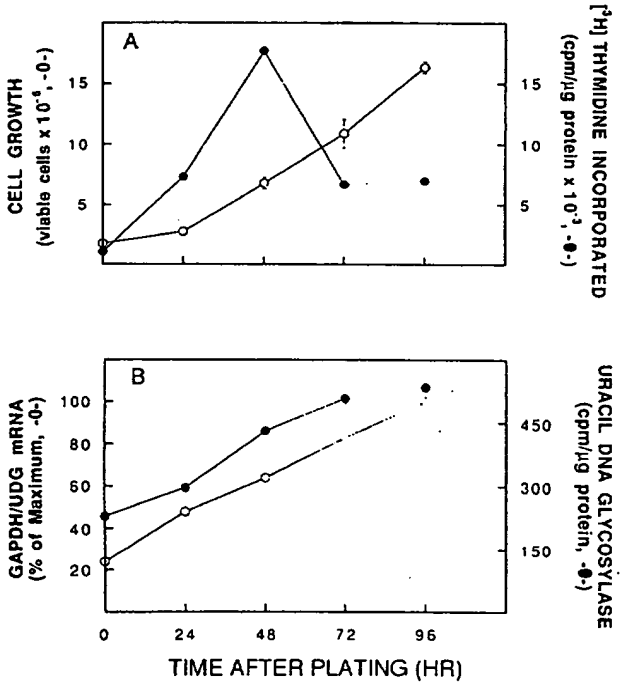


**Fig. 7.** Regulation of the GAPDH/UDG gene and protein in human lymphoblastoid cells. Molt cells were cultured as described in Materials and methods. Cell proliferation was initiated by replating cells from spent media at a lower density in fresh media. Transcriptional expression of the GAPDH/UDG gene was calculated as the percentage of maximal hybridization using the results described in Figure 6. Error analysis was performed as described in Figure 1.

gene should not be used in that capacity in studies in which the proliferative state of the cell did not remain constant.

Bloom's syndrome is an autosomal recessive human genetic disorder characterized by a number of cellular phenotypes (30). The Bloom's syndrome UDG in fibroblasts from four Ashkenazic Jewish and one African American patient was characterized by a specific immunological alteration in the glycosylase protein (24,25). As this aberration was also observed in Bloom's syndrome lymphoblasts, it would appear to be a germline defect (35). In this report, the regulation of the GAPDH/UDG gene and protein were examined in a non-transformed Bloom's syndrome cell strain, GM-05289, which was derived from a Japanese patient. Northern blot analysis revealed a single 1.6 kb mRNA whose expression was increased 4-fold during cell proliferation. This was equivalent to that observed in normal cells. It paralleled the induction of glycosylase enzyme activity as well as an increase in the level of GAPDH/UDG protein as detected by ELISA using the 42.08.07 antibody. However, using marker monoclonal antibody 40.10.09, no immunoreactivity was observed for the native uracil DNA glycosylase in either confluent or proliferating cells. This is the identical immunological alteration observed in all other Bloom's syndrome cells which have been examined. Thus, this observation extends this aberration in Bloom's syndrome to a third ethnic group providing further evidence that this immunological alteration is a general characteristic of this human genetic disorder.

These studies demonstrated the growth-dependent regulation of the GAPDH/UDG gene. However, further cell cycle experiments using frequent data points are required to determine the temporal sequence through which human cells express this gene in relation to DNA synthesis. As this gene encodes a multifunctional protein, the relationship between GAPDH/UDG gene expression and the regulation of each individual enzyme activity of this protein remains to be established. Immunocytochemical analysis demonstrated that the GAPDH/UDG protein was localized within or near the nucleus during cell proliferation (36). This correlated with the intracellular distribution of UDG enzyme activity. In normal human cells, UDG was regulated in the cell cycle in a defined relationship prior to the induction of DNA replication (3,4). In randomly proliferating Bloom's syndrome cells, the extent of UDG induction was comparable to that observed in normal human cells. However, in synchronized cells, enzyme activity was increased coordinate with DNA replication (30). This would indicate that a temporal alteration should exist with respect to the regulation of the GAPDH/UDG gene as well as similar perturbations in the increase in other enzyme activities which characterize this multifunctional protein. Accordingly, these results suggest that normal human cells may be used in future studies to examine the mechanisms through which a gene encoding a multifunctional protein is regulated in the cell cycle. Bloom's syndrome cells may be utilized in those investigations to probe the consequences which may arise when the cell cycle regulation of the GAPDH/UDG gene is altered.

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